REMARKS

Support for the amendments can be found in the Specification at:

Page	Line	Claim Paragraph	Amendment
5 6	34-37; 1-3	**4A**	Same target nucleic acid
6	15-16,	**4B**	multiple nodes
16	16-22;26-27	**4B**	multiple nodes
7	1-4	**4B**	genetic affinity
9	11-17	**4B**	genetic affinity
3	15-16	**4B**	genetic affinity
51	Fig 12& Table G	**4B**	genetic affinity
6	16-20	**4C**	At least 2 sequences
18	33-37	**4C**	fragmenting
22	16-22	**4C**	N=at least 7
12	1-2	**4C**	N=at least 7
7	9-11	**4F**	Representative nodes
-	-	**4F**	Renumbered
-	-	**4G**	Renumbered
See 4B		**4H**	Determine genetic affinity
25	1-10	**4J**	"
7	9-12	**4J**	"
51 58	Fig12 Table G	**10A**	To be consistent with Cl. 4 & 10
-	-	**10B &10C**	subsequence to simplify wording
-	-	**19**	To correct wording

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-	-	**23**	To remove "substantially"
-	-	**23**	To correct equation
	-	**39**	New claim
23; Fig 12	13-15	**39**	11 nodes
-	-	**40**	New independent claim
58-Table G	-	**41**	RNA or DNA
		42	New claim
58-Table G		42	Target sequence type
30	1-6	42	Target sequence type
		43	New claim
25	16-22	43	Inspecting nodes
23	12-15, Figure 12	44	At least 12 seq req'd for 11-node tree
-	Table B & Fig. 12	45 & 46	12 species sequences
22	21&Fig 9	45 & 46	Min. Length 7

Page 2 and following of the Office Action is reproduced below with Applicants' comments interspersed:

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DETAILED ACTION
Page 2
A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17 (e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 8/15/2008 has been entered Claims 30, 32-34, and 36-38 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected inventive group, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on 12/14/2007.

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Applicant's arguments, filed 6/30/2008, have been fully considered. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application
Applicants have amended their claims, filed 6/30/2008, and therefore rejections newly made in the instant office action have been necessitated by amendment.
Claims 4-10,19, 21,23-24, and 26-29 are the current claims hereby under examination.

Claim Objections
The objections to claims 4 and 26-39 have been withdrawn because of applicant's amendments to the claims.
The following is a newly applied objection:
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Claims 30, 32-34, and 36-38 are objected to because of the following
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informalities: The said claims have an inappropriate status identifier which should state "withdrawn." Appropriate correction is required.

Claim 30, 32-34 and 36-38 status identifiers now have been corrected and the error is regretted.

Claim Rejections - 35 USC § 112

Response to Arguments:
Applicant's arguments, filed 8/32/2007, with respect to the rejection under 35 USC 112 second paragraph have been fully considered and are persuasive because of applicant's amendment. Therefore the rejection has been withdrawn.

The Examiner is thanked for withdrawing the 35 USC 112 objections.

The following rejections have been necessitated by amendment:
The following is a quotation of the second paragraph of 35 U.S.C. 112:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.
Claims 4-10,19, 21,23-24, and 26-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.
Claim 4 (and all claims dependent therefrom), step A, comprises a step of "obtaining or creating a database of nucleic acid sequences of a homologous target RNA or DNA, from all organisms or viruses." which has been deemed as vague and indefinite. It is unclear as to how this target RNA or DNA is chosen or to what

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exactly may comprise the "homologous target." Furthermore, it is unclear as to the criteria used for selecting the target RNA or DNA from a sample that may comprise viruses.
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bacteria, mammalian tissue, etc. It appears from the claim wording that one target is selected, but unclear as to how that target is selected across varied organisms and viruses, etc. to ensure homology that can result in a database of nucleic acid sequences. Clarification via clearer claim wording is required.

The term homology has been removed from the claim as it might possible cause confusion. The phrase "same target nucleic acid sequences" is now included. One skilled in the art will recognize that this might be 16S rRNA sequences in the case of bacteria (specification pg 5 line 37 and pg 6 lines 1-3) or common genes encoded by genomic RNAs in the case of flaviviruses (specification pg 6 line 2-3 and page 32 lines 4-12).

The new recitations "multiple" and "eleven or more" distinguish the claims from the Ebersole reference which uses only one node. The amended phrase "establishes genetic affinity" may be clearer than "reflects genetic relationship"

In Claim 4 step B and new dependent Claim 39 "multiple" or "11 or more" has been inserted to place limits on the tree, in contrast to Ebersole which teaches a tree of 1 node. Eleven was chosen because that is how many nodes are shown in Figure 12, page 51 of the Specification.

Claim 4 (and all claims dependent therefrom) step C recites the limitation "each particular RNA or DNA subsequence of length n" in lines 1-2 of step C. There is insufficient antecedent basis for this limitation in the claim. It is unclear as to what subsequences said claim wording refers. Claim 4, step A, discloses obtaining target RNA or DNA from organisms or viruses, but does not disclose generating or creating any subsequences. Therefore, it is unclear as to what subsequences the wording in claim 4, step C refers. Claim 4 (and all claims dependent therefrom), step C has been

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deemed as vague and indefinite. It is unclear as how to obtain or what criteria is used for creating "each particular RNA or DNA subsequence of length N."

Claim 4, new paragraph C now recites expressly the step in which Applicants' subsequences are created. To provide a numerical minimum for N (for if N=0 or 1 Applicants' method clearly could not work); based on the example calculations in the Specification (Pg 31 lines 9-12), Applicants have also explicitly defined N as being at least 7.

Furthermore, it appears that N may be of any length, whereas it is unclear as to how the frequency of one single nucleotide leads to a determination of how it is characteristic of each node in the phylogenetic tree. Clarification via clearer claim wording is required.

In Amended Claim 4C, Applicants have now also explicitly defined N as being at least 7. It should be understood that this is merely to make the claim more clear and is not to be taken as narrowly critical to the invention.

Claim 4 (and all claims dependent therefrom), step E has been deemed as vague and indefinite. It is unclear as to how the derived plurality of signature probes necessarily determines the genetic affinity of twice the number of organisms or viruses.
It is unclear as to how many signature probes may be derived wherein a sample only comprised two organisms. For example, it appears that more than one signature probe
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may be derived per one characteristic signature sequence. Therefore, it is unclear as to how using several signature probes necessarily determine the genetic affinity of twice the number of organisms or viruses as probes used, such as in the instant case if only two organisms are present. Clarification via clearer claim wording is required.

The questioned phrase has now been cancelled from the claim 4, step F (formerly step 4E).

Claim 4 (and all claims dependent therefrom), step E comprises the wording "characteristic signature sequences," which has been deemed as vague and indefinite.
It is unclear as to what comprises these sequences or from where they are derived. It appears that these sequences are or maybe derived from the sequences stored in the database of step A.

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Clarification via clearer claim wording is required.

Claim 4 now defines the three databases used in the invention as: 4A nucleic acid database, 4C subsequence database and 4E signature database. This renders the objected-to phrase "characteristic signature sequences," unnecessary and it has been cancelled.

Claim 10 (and all claims dependent therefrom), step A, comprises the term "substantially," which is vague and indefinite and is not defined by the method of step A.
It is unclear as to what constitutes the term "substantially" and/or metes and bounds of said term. Therefore, one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. Clarification via clearer claim wording is required.

The objected-to "substantially" has been cancelled from Claims 10A, 23 and 29. It should be understood that that the recitations it modified are not narrowly critical, and those skilled in the Art should appreciate that they are entitled to some tolerance. See e.g The Court of Appeals for the Federal Circuit (CAFC 2002) held, in Verve, LLC v. Crane Cams, Inc.:"Expressions such as "substantially" are used in patent documents when warranted by the nature of the invention, in order to accommodate the minor variations that may be appropriate to secure the invention. Such usage may well satisfy the charge to "particularly point out and distinctly claim" the invention, 35 U.S.C § 112, and indeed may be necessary in order to provide the inventor with the benefit of his invention. In *Andrew Corp. v. Gabriel Elecs. Inc.*, 847 F.2d 819, 821-22, 6 USPQ2d 2010, 2013 (Fed.Cir.1988) the court explained that usages such as "substantially equal" and "closely approximate" may serve to describe the invention with precision appropriate to the technology and without intruding on the prior art. The court again explained in *Ecolab Inc. v. Envirochem, Inc.*, 264 F.3d 1358, 1367, 60 USPQ2d 1173, 1179 (Fed.Cir.2001) that "like the term 'about,' the term 'substantially' is a descriptive term commonly used in patent claims to 'avoid a strict numerical boundary to the specified parameter,'" quoting *Pall Corp. v. Micron Separations, Inc.*, 66 F.3d 1211, 1217, 36 USPQ2d 1225, 1229 (Fed.Cir.1995)."

Here, Applicants choose not to further delay prosecution of this Application, and prefer to leave this wording to reasonable interpretation by those skilled in the Art.

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Claim 23 (and all claims dependent therefrom), line 2, comprises the term "substantially," which is vague and indefinite and is not defined by the steps of claim 23. It is unclear as to what constitutes the term "substantially" and/or metes and bounds of said term. Therefore, one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. Clarification via clearer claim wording is required.

The objected-to "substantially" has been removed from Claims 10, 23 and 29. As discussed above, it should be understood that that the recitations it modified are not narrowly critical and must reasonably be given some tolerance by those skilled in the Art.

Step 4G combines former steps 4G and 4H for clarity and brevity.

New Claim 43 has been added to recite that Applicants simply examine the tree and see which are the closest. Some relevant support is at Page 20 lines 15-18; Page 25 lines 1-10; Page 7 lines 9-12 of Applicants' Specification.

Claim 25 (and all claims dependent therefrom), is being rejected because it comprises a broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim), which is considered indefinite.
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since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in Ex parte Wu, 10 USPQ2d 2031,2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required
feature of the claims. Note also, for example, the decisions of Ex parte Steigewald, 131 USPQ 74 (Bd. App. 1961); Ex parte Hall, 83 USPQ 38 (Bd. App. 1948); and Ex parte Hasche, 86 USPQ 481 (Bd. App. 1949). In the present instance, claim 26 recites the broad recitation wherein 15 sequences are used to determine the genetic

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affinity of at least 18 organisms, and the claim also recites the number of organisms or viruses whose genetic affinity might be determined is at least twice the number of probes, which is the narrower statement of the range/limitation.

Claim 25 has been cancelled without prejudice.

Claim 26 (and all claims dependent therefrom), has been deemed as vague and indefinite. It is unclear as to how the failure to detect a particular sequence results in increased confidence with which the genetic affinity of an organism or virus is determined. Clarification via clearer claim wording is required.

Claim 26 has been cancelled without prejudice.

Claim 27 (and all claims dependent therefrom), is being rejected because it comprises a broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim), which is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent
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protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in Ex parte Wu, 10 USPQ2d 2031,2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of Ex parte Steigewald, 131 USPQ 74 (Bd. App. 1961); Ex parte Hall, 83 USPQ 38 (Bd. App. 1948); and Ex parte Hasche, 86 USPQ 481 (Bd. App. 1949). In the present instance, claim 28 recites the broad recitation wherein more than one oligonucleotide or sequence is detected, and the claim also recites the number of organisms or viruses whose genetic affinity might be determined is at least twice the number of probes, which is the narrower statement of the range/limitation.

Claim 27 has been cancelled without prejudice.

Claim Rejections - 35 USC § 102-Maintained
The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:
(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined

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in section 351 (a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21 (2) of such treaty in the English language.

Claims 4-7 are rejected under 35 U.S.C. 102(e) as being anticipated by Ebersole et al. (US P/N).

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The claims are directed to a method for determining the genetic affinity of

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organisms or viruses in a test sample containing a nucleic acid comprising the steps of:

A) Obtaining or developing a bifurcating node phylogenetic tree that substantially reflects the genetic relationship between the organisms or viruses included in a database of sequences of the nucleic acid.

B) Identifying the extent to which each particular oligonucleotide or sequence of length N is characteristic of each node in the bifurcating node phylogenetic tree of genetic relationship.

C) Deriving a plurality of nucleic acid signature probes from a signature-database of signature sequences that are complementary to a portion of the nucleic acid sequence of the organism or virus such that the number of organisms or viruses whose genetic affinity might be determined is at least twice the number of probes used.

D) Hybridizing the signature probes to the nucleic acid obtained from the test sample under conditions where a detectable signal will be produced by signature probes that hybridize to the nucleic acid of the organism or virus.

E) Identifying signature probes which produce detectable signal.

F) Determining which nodes in the bifurcating node phylogenetic tree of genetic relationship produced detectable signal to identify the closest genetic relatives of the organism or virus in the test sample.

Ebersole et al. teaches at Col. 9, lines 35-45 that a phylogenetic Tree of Life was obtained and used for extracting sequences that represented the major microorganism domains, Bacteria and Archeae, which could be used as signature sequences for

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obtaining signature probes for testing for the presence of dechlorinating bacteria, which reads on method steps A-C.

Claims 4-7 are rejected under 35 U.S.C. 102(e) as being anticipated by Ebersole et al. (US P/N 6,797,817).

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The claims are directed to a method for determining the genetic affinity of [Page 8] organisms or viruses in a test sample containing a nucleic acid comprising the steps of:

A) Obtaining or developing a bifurcating node phylogenetic tree that substantially reflects the genetic relationship between the organisms or viruses included in a database of sequences of the

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nucleic acid.
B) Identifying the extent to which each particular oligonucleotide or sequence of length N is characteristic of each node in the bifurcating node phylogenetic tree of genetic relationship.
C) Deriving a plurality of nucleic acid signature probes from a signature-database of signature sequences that are complementary to a portion of the nucleic acid sequence of the organism or virus such that the number of organisms or viruses whose genetic affinity might be determined is at least twice the number of probes used.
D) Hybridizing the signature probes to the nucleic acid obtained from the test sample under conditions where a detectable signal will be produced by signature probes that hybridize to the nucleic acid of the organism or virus.
E) Identifying signature probes which produce detectable signal.
F) Determining which nodes in the bifurcating node phylogenetic tree of genetic relationship produced detectable signal to identify the closest genetic relatives of the organism or virus in the test sample.
Ebersole et al. teaches at Col. 9, lines 35-45 that a phylogenetic Tree of Life was obtained and used for extracting sequences that represented the major microorganism domains, Bacteria and Archeae, which could be used as signature sequences for obtaining signature probes for testing for the presence of dechlorinating bacteria, which reads on method steps A-C.

Ebersole *et al.* (Ebersole) uses the "tree of life" developed from 16S rRNA sequences for the sole purpose of identifying complete 16S rRNA entries that are phylogenetically diverse. Ebersole does not actually use the sequences obtained from RibisomalDatabaseProject (RDP) as signature sequences. Instead, he constructs alignments to identify variable regions (this is not actually necessary as the location of these variable areas is well known in the art) within which he searches for "unique sequences" (Col 9, lines 40-46). In the Applicants' invention there is no requirement that the tree which is utilized be developed from the molecule selected in step A of claim 4. Instead, as indicated in Step B of claim 4, the tree can be either obtained or developed. Thus, for example, the tree might be developed using gyrase B sequences and the target molecule might be 16S rRNA or the tree might simply be obtained from a scientific publication. In fact, the tree might be developed from many concatenated sequences. It is not essential to the

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manner in which the signature sequences of the instant invention are identified that the tree be based on the molecule that will ultimately be targeted.

In addition it should be recognized that the bifurcating phylogenetic tree is utilized in the instant invention to define genetic relationships among all the organisms/groupings included in the nucleic acid database that are being considered (Specification p. 6 lines 5-7). The availability of this information on genetic relationship is at the core of the applicant's invention. In contrast, Ebersole only teaches one group of organisms (the species *Dehalococcoides ethenogenes)* and never uses the Tree of Life to examine relationships between the organisms being considered (e.g. in their case known and unknown members of the species *Dehalococcoides ethenogenes*

Amended step A of claim 4 now recites a minimum limitation of two (e.g. multiple) on the number of nodes in the tree. In order to represent all the nodes in a tree of at least 11 nodes, it will be necessary to have at least 12 organisms represented in the database of nucleic acids (Specification p. 23 lines 11-15). That the number of nodes in a binary tree is one less than the number of organisms included is actually a mathematical characteristic of a binary tree. This distinguishes from Ebersole's use of a single node in his tree.

Note also Ebersole's inability to use viruses or non-rRNA as targets, in contrast to the present invention [See e.g. Ebersole Col. 2 middle and Col. 7 last].

> *Furthermore, Ebersole et al. teaches at col. 4, lines 55-67 and col. 5, lines 1-4, that sequence profiles, from which signature probes are derived, may be used to identify and subtype bacteria*

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with similar metabolic pathways. Therefore, a signature probe may be used to identify a dechlorinated bacteria and/or bacteria with similar metabolic pathways, such as subspecies of decholorinates. which further reads on step C) Deriving a plurality of nucleic acid signature probes from a signature-database of signature sequences thai are complementary to a portion of the nucleic acid sequence of the organism or virus such that the number of organisms or viruses whose genetic affinity might be determined is at least twice the number of probes used.

The examiner cites Ebersole *et al.* as teaching at Col 4, lines 55-67 and col 5, lines 1-4 that "the instant sequence profiles from which signature sequences are derived may be used to identify and subtype bacteria with similar metabolic pathways". This is, however, not always correct. For example, (Loffler, Sun, Li, & Tiedje, Applied Environmental Microbiology 66: 1369-1374 [2000]), point out that strains of *Dehalobacter, Desulfuromonas, Dehalospirillum, Desulfitobacter,* and even *Enterobacter* sp strain MS1 have "similar metabolic properties", e.g. the ability to dechlorinate (http://www.microbe.com/census_remediation_targets.html) tetrachloroethene (PCE) and TCE (trichloroethene). These strains are of very diverse genetic affinity, being even in different Orders. For example, *Dehalobacter* is a member of the Peptococcaceae Order and *Desulfuromonas* a member of the Desulfuromonadaceae Orderwhereas *Dehalococcoides* is in the Order Dehalococcoides. Thus, any sequence that uniquely targets *Dehalococcoides* in accordance with the signature sequence definition used by Ebersole *et al.* would *not identify* either known or unknown strains of these metabolically similar strains. Ebersole's argument, Col 5, lines 1-4 that "....SEQ ID NO:8 may be used to identify dechlorinators as well as for genomic sub-typing of species" does not hold for dechlorinators in general, only possibly for species and subspecies of the genus *Dehalococcoides ethenogenes.* In contrast, using the instant invention, one can include nodes in the tree that represent all of these groupings and

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hence the genetic affinity of *any* dechlorinator that was present can be identified, not just metabolically similar strains.

> *Ebersole et al. further teaches at col. 4, lines 55-67 and col. 5, lines 1-4 that the use of particular sequences may be used to identify decholorinators as well as for genetic sub-typing of species, which further reads on method step B) Identifying the extent to which each particular oligonucleotide or sequence of length N is characteristic of each node in the bifurcating node phylogenetic tree of genetic relationship.*

As clarified by the amended step B of Claim 4, the tree of relationship contains multiple nodes and establishes the relationship between the nodes. Ebersole only considers a single node and therefore does not use the tree in the manner of the instant invention. All of Ebersole's species, sub species, new dechlorinating bacteria, etc are *encompassed by a single node* which defines the species *Dehalococcoidse ethenogenes*. *Ebersole* does not consider how this node is related to other nodes in the tree, and in fact never utilizes the relationship information in the tree at all. Applicants Claim 4 step B recites multiple nodes.

> *Ebersole et al. further teaches at col. 2, lines 51-65, the use of signature probes in hybridizing to identifying sequences such that a signal is detectable, which reads on step D) Hybridizing the signature probes to the nucleic acid obtained from the test sample under conditions where a detectable signal will be produced by signature probes that hybridize to the nucleic acid of the organism or virus.*

Revised Claim 4 step D recites creating a signature database which contains what the specification of the instant invention refers to as signature sequences. It is believed that the examiner meant to cite step E. Assuming this to be the case, it should be noted that the phrase "signature sequence" as explicitly defined by Ebersole (column 5 lines 34-39) is distinctly different from that phrase as used in the instant application where "signature sequences are those test sequences having Qs above some criterion"

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(specification pg 7 line 1). Thus, the signature probes used by Ebersole are required to have high inclusivity and high exclusivity (See Ebersole's Column 2 lines 46-48; Col 5 lines 35-38; and other places including his one and only claim at col 78 lines 34-36). As a result, Ebersole's type of signature sequences only have diagnostic value at one node in the tree- the one they are designed to detect. In contrast, the signature sequences utilized in the powerful instant invention can have low inclusivity and exclusivity (Application p. 22 lines 1-8, Page 56, Table E), and still be useful. In addition, a single signature sequence (Application page 7 lines 1-4; page 23 lines 18-21,) can have signature value for more than one node. They are therefore not the types of signature probes referred to in current step G. Therefore, Ebersole does not read on step D of claim 4.

Moreover, in the context of his definition of signature sequence, Ebersole teaches finding sequences that are unique to organisms encompassed by the single node he is working with (the species complex known as *Dehaloccoides ethenogenes*). By emphasizing finding sequences that uniquely characterize this single node, Ebersole teaches away from the current invention. In contrast, the instant invention recognizes and takes advantage of the fact that individual sequences have signature value at widely differing levels for multiple nodes in the tree being used (specification page 6 lines 26-27; page 7 lines 1-4). For example, as shown at specification page 52 lines 13-17, the sequence AAAAAAAGAGGGUAG has widely different signature values at various nodes in the example tree. That this additional information might be useful is completely overlooked by Ebersole. This information is obtainable and made use of in the instant invention by the active role that the phylogenetic tree plays in the analysis.

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In contrast, Ebersole does not utilize a multiple node tree when seeking the essentially unique signature sequences required by his invention.

> *Ebersole et al. at col. 5, lines 34-39, col. 6, lines 31-34, col. 6, lines 58-67, and col. 7, lines 1-9 teaches using signature sequences for generating probes and defines the use of probes and hybridization as such that is consistent in the art, which produce detectable signals, which reads on step E) Identifying signature probes which produce detectable signal. Ebersole et al. teaches at col. 8, lines 38-40 that the sequences are useful for the identification of new dechlorinating bacteria, as well as for sub-typing strains of Dehalococcoides ethenogenes.*

All of the organisms considered by Ebersole *et al.*, whether they are new strains, previously unknown strains, or sub-type strains, are in fact encompassed by a single node in the phylogenetic tree. Ebersole knows before conducting the experiment that a positive result will be indicative of the presence of an organism represented by that single node, e.g. a member of the species *Dehalococcoides ethenogenes*. In contrast, in the instant invention it is unknown which node or nodes in the tree of multiple nodes (Claim 4 step B), preferably of at least 11 nodes (new dependent claim 39), will give a positive signal. Thus, depending what organism is in the test sample and which nodes give a positive signal, the organism may be identifiable at a very high level, e.g. a particular genus or just at a low level, e.g. a Gram positive organism. Hence, in general, genetic affinity with a particular node is determined rather than membership in a single specific predetermined grouping.

> *Furthermore, Ebersole et al. teaches at col. 9, lines 19-40 that sequences used for obtaining probes and closest or nearest organisms to these sequences were determined, which all read on step E) Determining which nodes in the bifurcating node phylogenetic tree of genetic relationship produced detectable signal to identify the closest genetic relatives of the organism or virus in the test sample.*

Ebersole never maps his results onto a phylogenetic tree because he in fact only considers one node (the species *Dehalococcoides*

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ethenogenes) and thus has no requirement for the tree other than to facilitate the original selection of sequences (the tree is actually not even essential to that process). In the instant invention, the signals, are mapped back to a tree of multiple (preferably11 or more) nodes in order to determine which node or nodes is responsible for the positive hybridization signal as now recited in step G of Claim 4 and in new Claim 43. This allows an organism encompassed by any of the nodes in the tree to be identified without prior knowledge of which node will encompass a bacterium or virus found in the test sample.

> *Ebersole et al. teaches claim 5 at col. 2, lines 50-59 wherein rDNA are used for obtaining probes, which reads the use of DNA for comprising signature probes.*
> *Ebersole et al. teaches claim 6 at col. 6, lines 58-67 wherein hybridization is taught with that which is consistent in the art wherein a hybridization step is done in solution, which reads on claim 6.*
> *Ebersole et al. teaches claim 7 at col. 13, lines 25-30 wherein it is taught that probes which generate a detectable signal are used, which inherently reads on a probe wherein the detection step utilizes radioactive labels, chemiluminescence, and/or fluorescence.*

Claims 5-7 and others are dependent on Claim 4 and are ipso facto allowable with Claim 4.

> *Response to Arguments:*
> *Applicant's arguments filed 6/30/2008 have been fully considered but they are not persuasive. Application/Control Number: 10/057,270 Art Unit: 1631 Page 11*
> *Applicant argues that Ebersole does not teach a method for analyzing what is in the sample, only whether or not a particular organism or type is present. Applicant's argument is not found persuasive because Ebersole et al. teach at col. 8, lines 38-40 that the sequences are useful for the identification of new dechlorinating bacteria, as well as for sub-typing strains of Dehalococcoides*

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ethenogenes, wherein the identification of new dechlorinating bacteria reads on analyzing what is in the sample and even if it" has not been encountered previously, not just if an organism is present

Applicant argues that by seeking to determine the presence of a specific group of organisms whose identity is known ahead of time. Ebersole teach away from the present invention. Applicant's argument is not found persuasive as Ebersole teach that "those 16S DNA gene sequences that were identified to be similar to the dechlorinating bacteria, Dehalococcoides enthenogenes DHE-195 (GenBank Accession No. AF004928), were aligned with selected 16s rRNA sequences extracted from the Ribosomal Database Project (Michigan State University) that were a representation of the major microorganism domains. Bacteria and Archeae in the Universal Phylogenetic Tree of Life. The sequences were aligned using MegAlign in DNAstar, using the default software parameters. From this alignment probable region for signature sequences were mapped. Furthermore, Ebersole teach the sequences are useful for the identification of new dechlorinating bacteria as well and therefore are not just specific.
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Ebersole's probes are designed to be specific to a single node on the phylogenetic tree as it is used in the instant invention as recited in Claim 4 step B as currently amended. That single node considered by Ebersole *et al.* is thought to represent the species *Dehalococcoides enthenogenes* and if it in fact it does, it is reasonable that it would encompass sub-types of the genus as well as members of the genus that have not previously been isolated. Together and separately, these organisms in fact constitute a single node on the tree. The Applicants' argument is that because Ebersole's invention only considers a single node, he is in fact "*seeking to determine the presence of a specific group of organisms whose identity is known ahead of time*". That is to say if a positive test is obtained then an organism belonging to the species cluster *Dehalococcoides enthenogenesis* present. In contrast the applicant's instant invention can identify signature probes that target at least half the nodes in a phylogenetic tree (Specification page 7 lines 9-11). As a result, a single set of probes can be designed that will allow determination of the

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genetic affinity of an organism within the context of as many nodes (multiple and preferably at least eleven) as are present in the Applicants' tree. Thus, a positive test is obtained in the applicant's invention it will not be known what the genetic affinity of the organism in the test sample is until the positive probes are examined in the context of the tree (revised claim 4 step G and new dependent claim 43). Moreover, it is also not clear at what phylogenetic level the organism in the test sample will be identified at until the data is examined in the context of the tree. This step (revised claim 4 step G) of examining positive results in the context of the tree is not needed nor is it taught by Ebersole, because he does in fact not use a tree of multiple nodes in the manner of the instant invention.

> *Applicant argues that Ebersole relies on the presence of a single sequence which must be completely complementary to the target, which teaches away by suggesting that one must have specific sequences for every target of interest. Applicant's argument is not found persuasive because applicant's claimed invention is based on specificity as in step E. Claim 4, step E derives signature probes from signature sequences, which will only be complementary to the target nucleic acid of the organisms which comprise the signature sequence. Therefore, the target nucleic acid of the organisms must have the signature sequence present.*

The Examiner's comments regarding step E (now step 4F) are correct. However, this reflects the fact that Applicant was not referring to the specificity during the hybridization step but rather was referring to the design stage when the probes are chosen. Ebersole seeks to identify as targets, "signature sequences" that are uniquely present in the 16S rRNA of the target node – *Dehalococcoides* and absent from the 16S rRNA of other organisms. See for example Ebersole Col 5, lines 35-40; Col 9, lines 43-46; Col 18, lines 8-12. Ebersole places great value on sequences that are present in all *Dehalococcoides* 16S rRNAs and not present in other organisms. These sequences are then used to design Ebersole's probes. This quest for

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very high node specificity of the signature sequences teaches away from the instant invention where signature sequences are selected very differently:

In the instant invention signature sequences include those that are informative about the genetic affinity of the organism or virus carrying the nucleic acid at widely varying levels. They are absolutely not required to have either perfect inclusivity or perfect exclusivity, and in fact, it can be best if they do not have either. Thus, p. 7 line 1 of the Applicant's application, indicates that the invention works to retain as signature sequences those test sequences having values of Qs (as defined in the Specification and in Claim 23 which is dependent on claim 10 which is in turn dependent on claim 4) above some criterion. In contrast to Ebersole, that criterion need not be 1 or even close to 1. For example, a signature sequence may occur in 90% of the organisms or viruses in one grouping, 70% of the organisms or viruses in another grouping and 45% in a third grouping. It would thus have a different Qs score for each grouping and each of these scores would be well below the perfect score of 1.0. (see p. 7, lines 1-4, and p. 22 lines 1-2 of the Applicant's application). That one can use such N-mers (subsequences) according to the present invention is extremely unexpected and non-obvious.

> *Applicant further argues that Ebersole teaches away because its very nature can only produce probes for specific groupings. Applicant's argument is not found persuasive because it is not commensurate in scope with the claimed invention. Ebersole's invention can detect known bacteria and also unknown or new bacteria, which reads on the instant claims which do not necessitate organisms from different groups to be present but rather a method for determining the affinity of organisms that are present. Therefore, a method which identifies previously unknown dechlorinating bacteria, but determines they are indeed dechlorinating bacteria, inherently determines the genetic affinity of the detected organism.*

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As discussed above, the probes used by Ebersole *et al.* can not determine the genetic affinity of all dechlorinating bacteria but rather only those that belong to the species *Dehalococcoides ethenogenes*. Moreover, if an organism that belonged to the species lacked the genes needed to exhibit the dechlorinating phenotype, it would be incorrectly defined as a dechlorinating bacterium by the method of Ebersole *et al.*

Setting the above issues aside, Ebersole can only detect "unknown" bacteria that specifically exhibit the dechlorinating phenotype whereas the instant invention determines genetic affinity without regard for any specific phenotype. In fact Ebersole *et al.* teach away from the notion that genetic affinity is being determined at all when on Col 3 lines 6-7 where it is stated "the invention further provides a method for IDENTIFYING a dechlorinating bacterial strain comprising...."

In the instant invention when an organism is encompassed by a node on the tree, it has genetic affinity with all other organisms encompassed by that node (Specification Page 8 lines 9-10. The number of nodes that can be addressed is limited by the number of nodes in the tree (preferably at least eleven) whose role in the process is now more clearly defined in amended step B of Claim 4. Because Ebersole does not use his tree in the novel way of the instant invention, his methods only allow decisions to be made regarding the presence of organisms belonging to a single node. Ebersole therefore teaches away from the notion that a single test system can be used to determine the genetic affinity of many organisms encompassed by different nodes (Specification page 7 lines 9-14).

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Applicant argues that the present application teaches that other nucleic acids can be used. Applicant's arguments are not found persuasive because they are not commensurate in scope with the claimed invention.

In fact, as claimed in currently amended Claims 10 and 19 which are both dependent on claim 4, the instant application recites that nucleic acids other than 16S rRNA or 16S rDNA can be used (see application p. 58, Table G; and also p30 lines 1-4,). Thus, for example, Applicants' method can be used with the genomic RNA of flaviviruses, which do not have a 16S rRNA. Ebersole *et al.* teaches away from this generality, by repeated emphasis on 16S rRNA and 16S rDNA (e.g. Col 1 lines 10-13, Col 2 lines 12-22; Col 4, lines 55-57; Col 5, lines 35-38, and others). In light of amended dependent claims 10 and 19, applicant believes that this argument is now clearly commensurate in scope with the claimed invention.

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Applicant argues that Ebersole uses the Tree of Life, i.e. phylogenetic tree for a different purpose wherein the present invention uses the tree to define the signature properties of every oligonucleotide of interest. Applicant's arguments are not found persuasive as Ebersole uses the Tree of Life to determine the signature properties of oligonucleotides from a dechlorinating bacteria species, wherein that specific bacteria was of interest.

Ebersole *et al.* use his tree of life for a single purpose, mainly to select diverse 16S rRNA sequences to compare his sequences of interest to as described at Col 9 lines 35-46. He aligns sequences from a single target group (*Dehalococcoides enthenogenes*) with "selected" other 16S rRNA sequences extracted from the Ribosomal Database Project database. This allows him to detect regions where searching for perfect signature sequences appears to be promising. The specific relationships between nodes on the tree are never utilized. Candidate signature sequences are then tested against the

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entire RDP database to find sequences that are "unique" to the target group. Thus, the tree is only being used to narrow the search for the unique sequences. In actuality, this step is not even necessary. One could simply search all possible N-mers of the desired length that occur in the target group against the whole RDP using a program such as Probe Match as described at Col 17, lines 23-29.

In contrast, in the instant invention the information embodied by the tree is integral to the procedure. In this regard, Step B, it is the information regarding relationships between nodes that matters and hence the tree may be developed from the sequences of the target nucleic acid molecules or simply used from an independent source. In step claim 4 step D (previously step C) the signature property of each N-mer at each node in the tree are examined. Each node that comprises the tree defines a grouping. These groupings are related Page 7 lines 3-4 of the application, by a child, parent, grandparent, etc. relationship. The tree is thus essential because it defines what organisms are encompassed by various nodes. Thus a node might represent a large grouping such as the Gram positive bacteria or it might be a single genus such as *Dehalococcoides*. The instant invention teaches the value of determining the signature properties of each N-mer under consideration at every node in the tree. In contrast, Ebersole *et al.* utilizes just a single node and hence teaches away from the present invention by failing to recognize the utility to be gained by incorporating the genetic affinity information inherent in the tree. Finally, in steps I and J of revised Claim 4 the tree is utilized to interpret the results. These steps are necessary because the inherent generality of the instant invention allows the genetic affinity of any organism or virus encompassed by the tree to be detected.

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Thus, after positive probes are detected one must utilize the genetic affinity information in the tree to properly interpret the results. In contrast, Ebersole *et al.*, only seeks to identify organisms associated with a single individual node and thus a positive result requires no further interpretation. Thus, step is not taught by Ebersole at the cost that they Ebersole *can* only identify the presence of organisms belonging to a single node.

Applicant argues that the instantly claimed invention relates the occurrence of each candidate signature to the various groupings in the tree.

Currently amended language of Claim 4, especially steps C and D as well as dependent claims 10 and Claim 23, now describe how this is done.

Applicant further argues that Step A recites a database which comprises the signature properties of large numbers of N-mers. Applicant's arguments are not found persuasive because they are not commensurate in scope with the claimed invention. Applicant's claim 4 step A only recites a database of nucleic acid sequences of a target DNA from all organisms that will be incorporated into the analysis, wherein there is no limitation as to how large or consequently how small the number of organisms

The examiner is correct in pointing out that the database in step A does in fact not include the signature properties of large numbers of N-mers. The claims have now been amended and the manner of constructing said database is now recited in Part D of newly clarified Claim 4. The Applicants respectfully request that the arguments previously erroneously made with reference to Step A of Claim 4 now be considered with reference to Part D of revised claim 4.

In essence those arguments were: ....revised Step D of Applicants' Claim 4 recites a database which comprises the signature properties of large numbers of N-mers. Ebersole *et al.* never create such a database and

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instead teach away from it by emphasizing the need to find and use small numbers of sequences that are as unique as possible to the target organisms. On p. 7 line 1 of the Application, it is pointed out that the current invention works to "retain as signature sequences those test sequences having values of Qs above some criterion". That criterion need not be 1 or even close to 1. For example, a signature sequence may occur in 90% of the organisms or viruses in one grouping, 70% of the organisms or viruses in another grouping and 45% in a third grouping. It would thus have a different Qs score for each grouping and each of these scores would be well below the perfect score of 1.0. (see p. 7, lines 1-4, and p. 22 lines 1-2 of the Applicant's application). That one can use such N-mers (subsequences) according to the present invention is extremely unexpected and non-obvious. The present invention teaches that such sequences with signature quality scores well less than perfect can in fact be very useful. For instance, in the example given above they contribute to the recognition of three distinct organism or virus groupings rather than just one. In order to take advantage of the information carried by such signature sequences, the present invention (p.7 lines 9-11) teaches the use of a "plurality" of such signature probes that target many separate groupings, not just one. Thus, an array of probes constructed from sequences in the signature sequence database can be constructed that can determine the genetic affinity of large numbers of diverse organisms regardless of any similarity of metabolic function. Ebersole *et al.*, teaches away from this conclusion by emphasizing that his bacteria must share "similar metabolic pathways". In fact, photosynthetic bacteria of various types do share similar photosynthetic pathways, but in terms of genetic affinity are not sufficiently related to be identified by a single signature probe of the

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Ebersole *et al.* type. So not only does Ebersole *et al.*, teach away from the current invention his suggestion is not generally valid

> *Applicant's claim 4 step A only recites a database of nucleic acid sequences of a target DNA from all organisms that will be incorporated into the analysis. wherein there is no limitation as to how large or consequently how small the number of organisms some of which may be known and some of which may not be known. Therefore, Ebersole, who teach that their invention can recognize both known dechlorinating bacteria and new, unknown. dechlorinating bacteria reads on the broad and reasonable interpretation of the instantly claimed invention.*

Revised step B of claim 4 now recites a minimum limitation on the number of nodes/organisms in the tree; e.g. "multiple" which is more than 1. In order to represent all the nodes in a tree of at least 11 nodes (new dependent claim 39), it will be necessary to have at least 12 organisms represented in the database of nucleic acids (Specification p. 23 lines 11-15). That the number of nodes in a binary tree is one less than the number of organisms included is actually a mathematical characteristic of a binary tree.

> *Applicant further argues that the retained signature sequences have values of Qs above some criterion. ..*
> *Applicant's arguments are not found persuasive as they are not commensurate in scope with the rejected claims as there is no calculation of a value for Q in the claim 4.*

Claims 23 and 29 recite the Qs values preferred to specify that neither high inclusivity nor high exclusivity is need by the present invention, in direct contrast to Ebersole. However, Claim 4 recites the distinctions discussed above which distinguish the invention over Ebersole.

> *Applicant argues that the instant invention teaches that such sequences can recognize, for example, three distinct organisms or various groupings rather than just one.*
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> *Applicant's arguments are not found persuasive because they are*

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not commensurate in scope with the claimed invention. Applicant's invention does not necessitate the recognition of various groupings but just organisms in the sample,

Step G of amended claim 4 identifies " the nodes in the bifurcating phylogenetic tree of genetic relationship that are represented by the signature probes that produced detectable signal, in order to determine the genetic affinity of the organism or virus in the test sample". This clarifies that in fact it is the nodes, e.g. groupings, which are recognized when pursuing the instant invention, not the identity of an organism in the sample. Genetic affinity is assigned by "identifying the organism or virus in the test sample as being contained within the most terminal node that is supported by one or more positive signature probes"(Specification-p. 35 lines 5-6). Each node represents a grouping (specification p. 3 lines 20-24) and the genetic affinity established can be with any node in the tree. The ability to recognize at least half the nodes in the tree (Specification p.7 lines 9-11) nodes (e.g. groupings) is essential to the instant invention. Thus, it is nodes that are recognized, not organisms. In contrast, Ebersole *et al.* teach away from the valuable generality of the present invention by devising a strategy that can only consider a single node.

Applicant further argues that the instantly claimed invention includes probes that determine genetic affinity without prior knowledge of what organism is present in a test sample. whereas Ebersole teach that the probes used target one specific group of bacteria. Applicant's arguments are not found persuasive as they are not commensurate in scope with the claimed invention. Ebersole does target specifically dechlorinating bacteria, but the probes used may also detect unknown dechlorinating bacteria. i.e new dechlorinating bacteria. In the invention taught by Ebersole. if the signature sequence is present in the target nucleic acid sequences of the organisms present, the characteristic signature sequences will hybridize, which reads on step E. The instantly claimed invention does not necessitate using a sample wherein there is a threshold number of known and unknown organisms present.

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Step B of revised claim 4 of the instant invention now provides a threshold of number of nodes (multiple- i.e. at least 2 and preferably 11 or more (new dependent claim 39) in the bifurcating phylogenetic tree. Thus, given that signature sequences will be found that "represent at least 50%, and typically more of the nodes," (Specification p.7, lines 9-11) at least 6 nodes will always be represented by the signature sequences of the instant invention whereas in Ebersole, only one node is represented. Thus, using the methods taught in the instant invention, a single set of probes based on signature sequences will always be able to recognize multiple groupings/nodes (at least 6) when a tree of 11 or more nodes is used, whereas the Ebersole will never be able to recognize more than one group. This reflects the powerful way in which the applicant's invention utilizes the tree, as now clarified in revised claim 4.

> *Applicant further argues that the signature sequences used by Ebersole are different from the signature sequences used by the instantly claimed invention. Applicant further states that the instantly claimed invention uses signature sequences*
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> *that are informative about the genetic affinity of the organism or virus carrying the nucleic acid.*
> *Applicant's argument is not found persuasive because the signature sequences used by Ebersole, if hybridized to a new bacteria, are informative about the genetic affinity of that bacteria to dechlorinating bacteria, which also inherently, because of the use of the Phylogenetic tree of life, is informative about the genetic affinity of that organism to other organisms.*

Ebersole does not use signature sequences to determine the affinity of an organism in a test sample to other groupings/nodes in the tree of life. In fact, genetic affinity is never mentioned by Ebersole . Instead, what Ebersole determines is identity (Col 1 lines 10-15; Col 2 lines 60-65; Col 3, lines 20-21; Col 8 lines 37-40; and others). If an organism in the test sample gives a positive signal it actually is identified as belonging to the single node

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defined by the signature sequences used, e.g. the species cluster *Dehalococcoides enthenogenes*. Ebersole is in effect determining organism presence, not genetic affinity. Presence because if a positive signal is obtained it is already known what the organism is. In the instant application genetic affinity and identity are actually distinguished (Specification p. 3 lines 23-25, 29-31). It is inherent to the instant invention that it is not known which node an organism in a test sample will have most affinity with until all the positive nodes are considered. That is to say, a positive result does not determine what is present. It remains necessary to examine where the positive nodes are in the tree as now clarified by Step G of revised claim 4. Moreover, the organism can belong to any grouping that is encompassed by the tree used (Specification p. 10 lines 7-10). Ebersole does not use his tree in this manner and thus does not teach Step G of Claim 4.

Regarding the tree of life- Ebersole mentions it explicitly at col. 9 lines 36-40 and implicitly at col. 17 lines 18-20 lines. In both cases, Ebersole's tree of life is solely used to facilitate the choice of a diverse sample of 16S DNA sequences. As discussed above, it is not even actually necessary for this purpose as the procedures outlined at col. 17 lines 23-28 could be applied to all possible candidate sequences. The genetic affinity of *Dehalococcoides enthenogenes* to other organisms in the tree of life is not considered in selecting the representative sequences. Instead, only the relationships to one another is considered in a very general way such that a set of sequences can be selected that are "representative of the major microorganism domains, Bacteria and Archaea in the Universal Tree of Life". Affinity of *Dehalococcoides enthenogenes*, or lack of it, to these representative organisms is not considered in the choice of 16S rRNA sequences to be

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considered. Thus, although genetic affinity is implicit to the tree of life, Ebersole *et al.*, are not using the tree of life in a manner that exploits the genetic affinity of *Dehalococcoides enthenogenes* to other organisms in any manner whatsoever.

> *Applicant argues that the examiner does not point to any part of Ebersole that may read on step F.*
> *Applicant's arguments are not found persuasive because as stated in the Final Office action mailed out 4/30/2008 Ebersole et al. further teach at col. 2, lines 51-65, the use of signature probes in hybridizing to identifying sequences such that a signal is detectable. which reads on step D) Hybridizing the signature probes to the nucleic acid obtained from the test sample under conditions where a detectable signal will be produced by signature probes that hybridize to the nucleic acid of the organism or virus.*
> *Ebersole et al. at col. 5, lines 34-39, col. 6, lines 31-34, col. 6, lines 58-67, and col. 7, lines 1-9 teach using signature sequences for generating probes and defines the use of probes and hybridization as such that is consistent in the art, which produce detectable signals.*

As stated above, Ebersole teaches something very different from the signature sequences used by Applicants. Ebersole seeks to identify as targets, "signature sequences" that are uniquely present in the 16S rRNA of the target node – *Dehalococcoides* and absent from the 16S rRNA of other organisms. See for example Ebersole Col 5, lines 35-40; Col 9, lines 43-46; Col 18, lines 8-12. Ebersole places great value on sequences that are present in all *Dehalococcoides* 16S rRNAs and not present in other organisms. These sequences are then used to design Ebersole's probes. This quest for very high node specificity of the signature sequences teaches away from the instant invention

> *Conclusion*
> *No claim is allowed.*

Applicants have claimed a powerful method of determining genetic affinity of a vast range of organisms and viruses. They have

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distinguished their method from Ebersole, which might possibly be thought of as a special one-node case of their general invention, by reciting multiple nodes, values of N and Qs, etc. The Claims have been distinguished from the Ebersole reference by recognizing theat subsequences used to design probes, "signature sequences" need not be unique to the node they characterize, (that is, high exclusivity and high inclusivity are not needed, in contrast to the repeated teaching of Ebersole. It is settled patent law that a sole reference must teach every feature for a claim to be rejected under 35 U.S.C. 102. Applicants now urge that they are entitled to a patent on their valuable invention under the mandate of 35 U.S.C. 101.

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jason Sims. whose telephone number is (571)-2727540.
If attempts to reach the Examiner by telephone are unsuccessful. the Examiner's supervisor, Marjorie Moran can be reached via telephone (571)-272-0720.
Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the Central PTO Fax Center. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993) (See 37 CFR § 1.6(d». The Central PTO Fax Center number is (571)-273-8300.
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/J Jason Sims/

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Any necessary (small entity) charges can be charged to USPTO Deposit Account 200336 of Technology Licensing Co. LLC. Correspondence may be addressed to Customer No. 26830.

The Examiner is especially invited to suggest allowable subject matter and to telephone Applicants' Attorney if that would expedite prosecution and disposal of this Application.

Respectfully submitted,

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